A NOTE ON THE CHEMOTAXIS OF OXYTRICHA ÆRUGINOSA.

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Oxytricha æruginosa, Wrzeniowski, is one of the *Hypotricha* occurring rather irregularly in laboratory cultures. Cultures of it, so far as my experience goes, are quite characteristic in the fact that they are distinctly brown in color, and decidedly more alkaline than the average cultures of *Paramecium*.

Jennings and Moore[1] found that this organism differed from the congeneric *Oxytricha fallax* by forming spontaneously dense aggregations like those of *Paramecium*. On the other hand they found that unlike *Paramecium* it was not positive to carbon dioxide or indeed to any acid. They found that if both forms were mounted on the same slide according to Jennings's now well-known method, they would form separate aggregations, and that the members of each species would pass freely through collections of the other without being in any way detained. It is obvious therefore that the cause of aggregation in the two forms must be different.

Several years ago I made an attempt, at the suggestion of Professor Jennings, to learn somewhat more about this difference. Unfortunately I was unable at that time to complete the work, and I have not since been able to secure the necessary cultures of *Oxytricha*.[2] It seems wise therefore to publish the results so far obtained even though they may seem fragmentary.

The spontaneous aggregations of *Oxytricha* on slides or even in small open dishes are remarkable in being much more dense than those of *Paramecium*, in being much more sharply circumscribed, and in expanding much less rapidly. In certain mixed mounts of *Paramecium* and *Oxytricha* I found that, probably by mere coincidence, both forms congregated in the same area. In such cases at first the area inhabited by both was the same, ex-

[1] *American Journal of Physiology*, Vol. 6, 1902, pp. 242–243.

[2] For the rest of this paper *Oxytricha* is to be considered as meaning *O. æruginosa*.

cept that *Paramecium* swam further out, possibly due to the greater momentum caused by their larger body and faster rate of motion. But soon *Oxytricha* began to shun the center of the drop, repelled by the carbon dioxide exuded by the *Paramecia.* But they were held by their own attractive excretion in a ring around the outside of the area. Within thirty minutes however the *Oxytricha* had moved so far outward that the *Paramecia* were entirely separated from them; shortly afterward the aggregation of *Oxytricha,* due undoubtedly to its attenuated character, broke up, while the *Paramecia* were still densely crowded together. In a second similar group, where however the relative number of *Paramecia* was much smaller, the two organisms remained evenly distributed throughout the drop, and the area was extended at the same rate for both of them. Why this difference? Very likely because the smaller number of *Paramecia* acted less rapidly in making the center of the drop uninhabitable for *Oxytricha,* while the *Oxytricha* in moving outward, by their own excretion of carbon dioxide kept the whole area acceptable to *Paramecium.*

Paramecia in culture water, after aëration, react positively to distilled water or weak acid. When in distilled water they react positively to weak acid but negatively to stronger acid or to alkali. *Paramecium* therefore usually seems to seek an optimum region, weakly acid in reaction. (Of certain exceptions to this I hope to speak in another paper.) The relation of this to the general life activities of *Paramecium* has often been pointed out by Jennings.

A culture of *Oxytricha,* aërated in similar fashion, reacts quite differently. If such an aërated culture is placed under a cover glass on a slide, and a drop of distilled water is introduced, the center of this drop remains free from *Oxytricha.* Such as swim into the edge of the drop give the typical motor reaction, long before they reach the center, and then swim outward, but only for a short distance; for there exists now also an outer boundary bringing forth the same reaction. The result is that there is soon formed a ring-like aggregation, quite like that of *Paramecium* in presence of a drop of stronger acid. If such an aggregation is left undisturbed for some time, the *Oxytricha* usually soon begin to collect in some one portion of the ring, and within five or six minutes form there a very dense aggregation.

When similarly aërated cultures are tested with alkaline solutions it is found that with very weak alkalies, such as $n/1,000 - n/1,500$ KOH, the results were quite parallel with those with distilled water. That is, there was a ring-like aggregation around the edge of the drop. When the culture was made slightly acid, and then a drop of hydrant water (alkaline) was introduced, there was usually a more or less distinct ring formation, occasionally a complete negative reaction. The hydrant water used was decidedly more alkaline than the ordinary cultures of *Oxytricha.* That *Oxytricha* forms a ring around such a drop, might make one suppose that here we find an optimum of slight alkalinity for *Oxytricha.* But such proves not to be the case, at least not in so simple a form. Many experiments showed that so far as my cultures went, *Oxytricha* was always negative to stronger KOH ($n/200$) often with formation of a ring, and without exception wholly indifferent to weaker KOH. There was never a gathering within the drop no matter what dilution was used.

It seems then that there is really no optimum strength of alkali in which *Oxytricha* will gather. In those cases, just mentioned, where there is a ring formed, we must rather suppose that the alkali plays no part except to repel, but that the water acts as diluent to some other substance present in the culture, attractive to *Oxytricha*, but present in super-optimum amount.

In cultures made slightly acid, *Oxytricha* reacts to weak alkali either by collecting in it (rarely), or by forming a ring around it, depending on the strength of the alkali. Here again it is probably not an optimum condition somewhere in the alkali that causes the result, but rather a reduction to an optimum amount of some other substance present in the culture medium, coupled with a strongly negative reaction toward the acid. For if instead of a weak alkali we use a drop of culture liquid, which is normally weakly alkaline, we find an immediate and strong positive reaction in all cases. It is then not alkalinity but an unknown factor that produces this result. That alkalinity is not the factor is shown by further experiment.

In one culture it was found that *Oxytricha* when exposed in culture water to a drop of distilled water reacted purely negatively, and this whether the culture was aërated or not, or

whether the distilled water was aërated or not. Many repetitions always gave the same result. It seemed possible that here was a culture with optimum alkalinity. If so an increase in its alkalinity ought to produce ring formation around a drop of distilled water. To test this the culture was made rather strongly alkaline by addition of $n/100$ KOH, and to a mount a drop of distilled water was added. But the result was as before. In every case this one race, even when the culture was made many times more alkaline than in its natural state there was still a purely negative reaction toward distilled water. There was evidently an optimum condition in this culture, but it was not an optimum of alkali.

Some other cultures varied from this by showing only an incipient tendency toward ring formation with distilled water, in others again *Oxytricha* soon approached very close to the center of the drop. It seems obvious therefore that various cultures differ widely as to their approach to an optimum state, but that this variation is not, primarily at least, correlated with alkalinity. It seems plain to me, that the reactions of cultures to distilled water are due not to alkalinity but to the effect of some other yet unknown condition. Distilled water represents to *Oxytricha* not merely a transition stage between weak alkali and weak acid. If it did one might expect *Oxytricha* (always, when in culture solution, negative to even very weak acid) when placed in weak acid, to react positively to distilled water. A culture was made acid and aërated, then a drop of distilled water introduced on a mount. In some cases there was indifference, in a few there *was* a positive reaction, but in by far the greatest number of cases the organisms gave a negative reaction toward the distilled water, as most alkaline cultures would have done, sometimes with ring formation.

The same acidified culture however reacted positively to a drop of its own unacidified culture water.

It seemed obviously desirable to find out something of this unknown constituent leading to these results. Most of the experiments seemed to show that such a substance was present usually in more than optimum amount, hence the frequent occurrence of ring formations around diluents. It should be noticed

that in spite of our knowledge of the lack of reaction of *Oxytricha* to carbon dioxide, I took the precaution of aërating the cultures in all the above experiments, except where otherwise stated. The reason for this precaution lies in the following results.

A portion of an *Oxytricha* culture was taken and thoroughly aërated by squirting it repeatedly into a watch glass from a pipette. A mount was immediately made and a drop of non-aërated liquid from the same culture introduced. The result was a strong positive reaction toward this non-aërated drop. To an entirely similar mount a drop of *aërated* culture water was added. The organisms were entirely indifferent, swimming in and out of the drop, with no sign of reaction. Thirdly, a non-aërated mount was now made, and a drop of aërated culture water was added. The drop remained free from *Oxytricha*, which reacted negatively at its outer edge. If to a non-aërated mount a non-aërated drop was added, there was again indifference. These experiments were repeated many times, always with the same results. It is evident therefore that a non-aërated culture contains some substance toward which *Oxytricha* reacts positively, and that this substance can be removed by aëration. It must therefore be either volatile, gaseous or easily oxidized.

But an *aërated* culture will also react negatively to hydrant water or to distilled water, often with ring formation. That such ring formation occurs with distilled water shows that what happens here is due to another, non-volatile substance present in the culture, toward which *Oxytricha* also reacts positively at proper concentration. This second substance also seems to be present usually in super-optimum amount. This second substance may sometimes be the alkali. That it is not always such is of course well shown by the case mentioned above, where a culture, after addition of much KOH and of aëration, still remained purely negative to distilled water. This experiment also shows that it is not merely a matter of osmotic pressure.

An interesting experiment in this connection is that in which *Oxytricha*, previously gathered into hydrant water by electrotaxis, were mounted and a drop of slightly acidulated culture water added. There was quickly formed a dense ring around the drop, which was long maintained. It was evident that here

one or both the unknown substances attracted the organism, while the acid above a certain strength repelled it. The *Oxytricha* under the influence of these opposite forces, was held along a line where the two balanced each other. According to Jennings, an exactly similar phenomenon occurs in *Paramecium*, when exposed to a mixed solution of acid and table salt.

It appears then that we have here at least two unknown substances, one volatile, the other not, affecting the chemotactic reactions of *Oxytricha*. The presence of two such substances, whatever may be their origin, helps to explain away a difficulty which I think has been overlooked. In the ring formation so commonly occurring, the *Oxytricha* are at first distributed equally around the circle. They are held within it, so we suppose, because some substance is there in optimum quantity. As the drop, say of distilled water, diffuses this optimum area moves outward, and the organisms move with it. However, if undisturbed, the *Oxytricha* will soon be congregating in some one portion of the circle and forming a dense mass, entirely similar to the ordinary spontaneous aggregations. Now on the supposition that there is only one attractive substance concerned, such aggregation in one part of a circle which is supposed to uniformly contain an optimum amount of this attractive substance is hard to understand. But if we suppose that in the dilution of one of two attractive substances to an optimum strength the second substance is reduced below its optimum, then it is easily seen that the meeting of two or three *Oxytricha* in some part of the circle would soon tend to bring this second substance to its optimum strength in that part of the circle. Then naturally any further individuals entering this smaller area would be retained there, in similar manner to that by which they are retained in the circle as a whole.

I can only add that there is some evidence that these substances are formed with the development of a colony, and that the character of the chemotactic reaction itself is altered in the course of such development. A culture of *Oxytricha* rejuvenated by addition of fresh hay and water, acted very anomalously. The creatures were positive to distilled water, as well as to $n/100$ HCl. But two days later all this had changed, and the reactions to both substances were negative.

In older colonies the attractive substances seem to develop far above the optimum amount. In such culture water the amount can be again reduced by filtering and allowing the filtrate to stand over night in an open dish. Whether such reduction is by volatilization or oxidation, or by both, I do not know. Certain it is that to such a liquid *Oxytricha* taken from the same culture from which it came, react positively.

Ordinarily a mount from any culture will be indifferent toward its own filtered culture water, if both remain unaërated. But if from a culture a highly concentrated mass of individuals are taken and kept for a few hours in a small watch glass, and then mounted, they will give a strong positive reaction toward a drop of water from their own original culture. Evidently here a close aggregation of individuals has supercharged the water, hence a positive reaction to the original culture solution of lesser strength.

Peculiarly, of two colonies of *Oxytricha* each, unaërated, was negative to the culture water of the other, but when aërated it became positive, the introduced drop in both cases being unaërated. This again points to a marked difference between cultures, as several other facts do. To this phase of the subject I expect to recur in another paper.

In a search for a clue to the unknown attractive substances, I tried many organic acids, sugar, urea, acetic aldehyd, potassium cyanide, ammonia, and a few others, but always with purely negative reactions. The possibilities of the case are of course nearly endless, but should opportunity offer, I would wish to experiment with organic sulphur compounds, and with compounds of ammonia, and this purely on the empiric basis of the odors of *Oxytricha* cultures.

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